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FUNDAMENTAL STUDIES IN THE MOLECULAR BASIS OF LASER INDUCED RETINAL DAMAGE

Final Report

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FOREWORD

In conducting research using animals, the investigator(s) adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (NIH Publication No. 86-23, Revised 1985).

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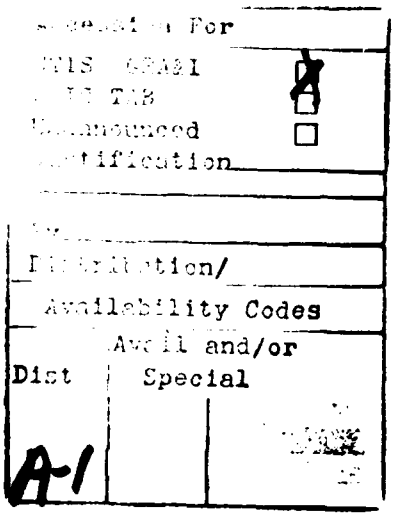

This is a comprehensive final report of the support for our research on "Fundamental Studies in the Molecular Basis of Laser Induced Retinal Damage" received by the U. S. Army. This research extended from 1979 through 1987 was covered by two contracts numbered DAMD17-79-C-9041 and DAMD17-85-C-5136. During this period great progress was made in developing new and powerful approaches to help us understand the molecular basis of laser induced retinal damage. This progress will be reviewed in this report.

In the initial phases of the research we focused on tunable laser resonance Raman spectroscopy to investigate the molecular basis of retinal damage problems. A principal feature of this technique is the capability to selectively enhance with appropriate tunable laser frequencies the structurally and environmentally sensitive vibrational spectra of various components of photoreceptor cells. One of the most attractive experimental features of tunable laser resonance Raman spectroscopy is the flexibility with which this spectroscopic tool allows the study of any form of matter from single crystals to dilute solutions to whole tissue. In essence, all that is needed to obtain the selective enhancement of the vibrational Raman spectrum of a chromophore embedded in a tissue is to scatter a laser off the tissue at an appropriate frequency within the electronic absorption of the chromophore and focus the scattered radiation into a monochromator. A principal problem in Raman spectroscopy is fluorescence which occurs at longer wavelengths (lower energies) than the incident laser wavelength. This is the same region where the strongest Raman scattering is detected, and since fluorescence is more intense than Raman scattering it usually obliterates the Raman spectrum when it

occurs. Early in our research efforts we made important advances to overcome this problem. These efforts were largely quite successful. Specifically, besides the rather simple, partially successful approach of moving the excitation laser frequency to lower energy, we introduced techniques such as modulating the incident laser frequency and using coherent scattering processes such as coherent anti-Stokes Raman spectroscopy or coherent Stokes Raman spectroscopy.

Using these techniques we were able to obtain crucial information on the oil droplets of red-eared swamp turtle cones. From these studies we began to investigate the very rapid bleaching of molecules involved in oil droplets. For these studies we measured picosecond bleaching effects over a broad range of illumination conditions. The results indicated that under certain conditions the carotenoids involved in oil droplets could act as a protection for very rapid pulsed lasers in the subpicosecond domain. At the time these results were obtained such lasers simply did not exist. Now, with the presence of femtosecond lasers the utility of such carotenoid filters should be further investigated.

In addition to the above practical results, fundamental information on the origin of the extremely large optical densities observed in oil droplets were obtainded. Excitation profiles of these oil droplets gave insight into how excitation, interactions and aggregation of carotenoid molecules could couple to give the unique optical densities of oil droplets. With this fundamental information we were able to extend our measurements to understand the origin of laser induced effects on the oil droplets. Specifically, we discovered that new molecular species arose from the laser irradiation and these

species were fluorescent. The fluorescence once characterized could then be used to detect the onset of damage in the oil droplets with great sensitivity.

In addition to applying laser spectroscopy to investigate damage mechanisms we also developed sensitive assays for the biochemical amplification steps in photoreceptors. These assays were used to assess the integrity of the photoreceptor cell under various illumination conditions. The assays centered around analyzing specific ions such as protons and how they were altered by light effects. For other alterations in the chemistry of the cell we applied ion microscopy and obtained sensitive detection of the calcium morphology in the photoreceptor under a variety of illuminations. Using similar instrumentation we were also able to investigate the elemental composition of melanin granules and their alteration with illumination.

This research led to the discovery that there are a series of anionic activators of visual cells. These anionic activators turn on, in the dark, the enzymatic processes which are usually stimulated by light. Among these anionic activators is fluoride, the important additive in dental care. As a result of this discovery of anionic activators visual sensitivity and excitation it may be possible to modulate such sensitivity by the development of new technologies to safely insert such activators into the living eye.

Another major advance in the general area of cytochemistry was the development of a staining method which allowed the direct observation with light microscopy of actin filaments in rod outer segments. This discovery allowed us to view these important actin filaments in live cells under physiologically relevant experimental conditions and

led to the development of new methods to probe pathological and damaged conditions in visual photoreceptor cells.

Our research also saw the first application of femtosecond lasers to the visual system. This research is giving new insights into how these ultimate laser sources interact with biological tissue in general and with the visual system in particular. In addition the research included the first application of Raman microprobe measurements to questions of laser induced retinal damage and we obtained data demonstrating rapid mechanical motions in vertebrate photoreceptors. Such rapid mechanical motions which parallel electrophysiological responses in the cell may lie at the very basis of photoreceptor function. In addition, we applied for the first time acoustic microscopy to investigate these questions of photoreceptor mechanics and the results successfully elucidated the morphology of the mechanical changes in the photoreceptor. Laser damage mechanisms need to be evaluated in terms of this new information of photoreceptor mechanics.

Finally, in order to fundamentally understand the interaction of light with photoreceptor cells a detailed study of the excited state properties of the retinylidene chromophore and related molecules was undertaken. Several spectroscopic techniques have been applied to investigate the photophysical and photochemical properties of the retinylidene chromophore. But few nonlinear optical techniques have been used. Such methods can yield crucial information. Second harmonic generation (SHG) is the lowest order nonlinear optical responsible for the generation of light at the second harmonic-frequency. Due to symmetry considerations, the SHG is forbidden in an isotropic medium in the electric dipole approximation, but allowed at

the surface where the inversion symmetry is broken. This attribute can be used to probe adsorbed monolayers at surfaces of membranes with high spatial and temporal resolution. Of great importance is the fact that this technique has been demonstrated to have submonolayer sensitivity and using the surface second harmonic generation technique directly on a Langmuir-Blodgett trough it was possible to measure the second-order molecular hyperpolarizabilities.

During the past year we have been able to apply SHG to compare the second harmonic properties of monolayers of retinal, retinylidene n-butylamine Schiff base (NRB) and protonated NRB (PNRB). The results have yielded new insight into the structure and dipolar properties of these molecules of great importance to vision.

In addition to the above, we have been able to apply a whole variety of other non-linear spectroscopies to the visual process. Of special interest are our four-wave mixing experiments on retinylidene chromophores which are free in methanolic solution or bound in the protein pocket. These experiments have been performed using a dye laser of correlation time of one hundred femtoseconds. An ultrafast phase relaxation process which a dephasing time less than 100 fsec was observed for both the free and bound chromophores. The results indicate that the protein matrix plays a minor role in the initial femtosecond dephasing process of the chromophore.

Thus, in conclusion the research supported under this contract has investigated the fundamentals of light-induced effects in vision using a variety of important and sensitive probes developed in our laboratories. The results obtained have led to insights of significance in most aspects of the visual process from femtosecond light

absorption, to photoreceptor mechanics and chemistry to the protective effect of oil droplets and the characteristics of the pigment epithelium. The new associations with the Hebrew University Hadassah hospital I hope to establish in the coming years will surely fully utilize these important advances and sensitive tools developed at Cornell to investigate the fundamental basis of laser induced retinal damage.

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